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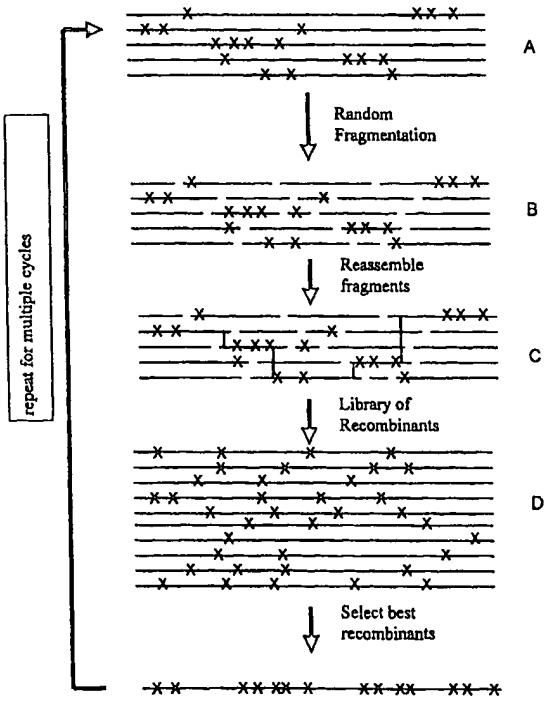
(54) Title: GENETIC VACCINE VECTOR ENGINEERING

(57) Abstract

This invention provides methods of obtaining vaccines by use of DNA shuffling. Through use of the claimed methods, vectors can be obtained which exhibit increased efficacy for use as genetic vaccines. Vectors obtained by using the methods can have, for example, enhanced antigen expression, increased uptake into a cell, increased stability in a cell, ability to tailor an immune response, and the like.



WHAT IS CLAIMED IS:

1. A multicomponent genetic vaccine comprising two or more genetic vaccine components selected from the group consisting of:

a component that provides optimal antigen release;

a component that provides optimal production of cytotoxic T lymphocytes;

a component that directs release of an immunomodulator;

a component that directs release of a chemokine;

a component that facilitates binding to, or entry into, a desired target cell type;

a component that directs antigen peptides derived from uptake of an antigen into a cell to presentation on either Class I or Class II molecules.

2. The genetic vaccine of claim 1, wherein each component is present on a separate vector.

3. The genetic vaccine of claim 1, wherein each component is present on the same vector.

4. The genetic vaccine of claim 3, wherein the vector is assembled by assembly PCR using as templates DNA fragments including a) a fragment which contains the first optimized recombinant genetic vaccine component and b) a separate DNA fragment which contains the second optimized recombinant genetic vaccine component.

5. The genetic vaccine of claim 1, which comprises a component designed for optimal antigen release that improves binding to, and uptake of, the genetic vaccine to target antigen-expressing cells.

6. The genetic vaccine of claim 5, wherein the target antigen-expressing cells are selected from the group consisting of myocytes and epithelial cells.

7. The genetic vaccine of claim 1, wherein the component confers optimal binding to, and uptake by, a target antigen presenting cell.

8. The genetic vaccine of claim 7, wherein the target antigen presenting cells are selected from the group consisting of dendritic cells, monocytes/macrophages, and Langerhan's cells.

9. The genetic vaccine of claim 1, wherein the component directs antigen peptides derived from uptake of an antigen into a cell to presentation on either Class I or Class II molecules.

10. The genetic vaccine of claim 9, wherein the component directs antigen peptides to presentation on Class I molecules and comprises a polynucleotide that encodes a protein selected from the group consisting of tapasin, TAP-1 and TAP-2.

11. The genetic vaccine of claim 9, wherein the component directs antigen peptides to presentation on Class II molecules and comprises a polynucleotide that encodes an endosomal or lysosomal protease.

12. The genetic vaccine of claim 1, wherein the desired target cell type is a dendritic cell or a Langerhans cell.

13. The genetic vaccine of claim 1, wherein the vaccine comprises:

a component for optimal antigen release;

a component optimized for CTL activation via dendritic cell presentation of antigen peptide on MHC Class I;

a component optimized for release of IL-12 and IFNγ from resident tissue macrophages; and

a component optimized for recruitment of T_H cells to an immunization site.

14. The genetic vaccine of claim 1, wherein one or more of the components is obtained by a method comprising:

(1) recombining at least first and second forms of a nucleic acid which can confer a desired property upon a genetic vaccine, wherein the first and second forms differ from each other in two or more nucleotides, to produce a library of recombinant nucleic acids; and

(2) screening the library to identify at least one optimized recombinant component that exhibits an enhanced capacity to confer the desired property upon the genetic vaccine.

15. The genetic vaccine of claim 14, wherein the method used to obtain one or more of the components further comprises:

(3) recombining at least one optimized recombinant component with a further form of the nucleic acid, which is the same or different from the first and second forms, to produce a further library of recombinant nucleic acids;

(4) screening the further library to identify at least one further optimized recombinant component that exhibits an enhanced capacity to confer the desired property upon the genetic vaccine; and

(5) repeating (3) and (4), as necessary, until the further optimized recombinant component exhibits a further enhanced capacity to confer the desired property upon the genetic vaccine.

16. The genetic vaccine of claim 14, wherein the first form of the nucleic acid comprises a first member of a gene family and the second form comprises a second member of the gene family.

17. The genetic vaccine of claim 14, wherein the optimized recombinant component is backcrossed by:

recombining the optimized recombinant component with a molar excess of one or both of the first and second forms, to produce a further library of recombinant nucleic acids; and

screening the further library to identify at least one optimized recombinant component that exhibits a further enhanced capacity to confer the desired property upon the genetic vaccine.

18. The genetic vaccine of claim 16, wherein the first member of the gene family is obtained from a first species of organism and the second member of the gene family is obtained from a second species of organism.

19. The genetic vaccine of claim 14, wherein the genetic vaccine comprises DNA.

20. The genetic vaccine of claim 14, wherein the genetic vaccine comprises RNA.

21. The genetic vaccine of claim 14, wherein the genetic vaccine comprises a viral vector or a plasmid vector.

22. The genetic vaccine of claim 21, wherein the viral vector is selected from the group consisting of adenoviral, retroviral, papillomavirus, adenoassociated, and herpes viral vectors.

23. A method of obtaining a genetic vaccine component that confers upon a genetic vaccine vector an enhanced ability to replicate in a host cell, the method comprising:

creating a library of recombinant nucleic acids by subjecting to recombination at least two forms of a polynucleotide that can confer episomal replication upon a vector that contains the polynucleotide;

introducing into a population of host cells a library of vectors, each of which contains a member of the library of recombinant nucleic acids and a polynucleotide that encodes a cell surface antigen;

propagating the population of host cells for multiple generations; and

identifying cells which display the cell surface antigen on a surface of the cell, wherein cells which display the cell surface antigen are likely to harbor a vector that contains a recombinant vector module which enhances the ability of the vector to replicate episomally.

24. The method of claim 23, wherein the cells which display the cell surface antigen on a surface of the cell are identified by flow cytometry-based cell sorting.

25. A method of obtaining a genetic vaccine component which confers upon a vector an enhanced ability to replicate in a host cell, the method comprising:

creating a library of recombinant nucleic acids by subjecting to recombination at least two forms of a polynucleotide derived from a human papillomavirus that can confer episomal replication upon a vector that contains the polynucleotide;

introducing a library of vectors, each of which contains a member of the library of recombinant nucleic acids, into a population of host cells;

propagating the host cells for a plurality of generations; and

identifying cells that contain the vector.

26. The method of claim 25, wherein the polynucleotide comprises either or both of the human papillomavirus E1 and E2 genes.

27. A method of obtaining a genetic vaccine component that confers upon a vector an enhanced ability to replicate in a human host cell, the method comprising:

creating a library of recombinant nucleic acids by subjecting to recombination at least two forms of a polynucleotide that can confer episomal replication upon a vector that contains the polynucleotide;

introducing a library of genetic vaccine vectors, each of which comprises a member of the library of recombinant nucleic acids, into a test system that mimics a human immune response; and

determining whether the genetic vaccine vector replicates or induces an immune response in the test system.

28. The method of claim 27, wherein the test system comprises human skin cells present as a xenotransplant on skin of an immunocompromised non-human host animal.

29. The method of claim 28, wherein the host animal is a mouse.

30. The method of claim 28, wherein the host animal is transiently immunocompromised.

31. The method of claim 27, wherein test system comprises a non-human mammal that comprises a functional human immune system and replication is detected by determining whether the animal exhibits an immune response against the antigen.

32. The method of claim 31, wherein the non-human mammal that comprises a functional human immune system is obtained by introducing into an immunodeficient non-human mammal one or more of a human fetal tissue selected from the group consisting of liver, thymus, and bone marrow.

33. A method of obtaining a recombinant genetic vaccine component that confers upon a genetic vaccine an enhanced ability to induce a desired immune response in a mammal, the method comprising:

(1) recombining at least first and second forms of a nucleic acid which comprise a genetic vaccine vector, wherein the first and second forms differ from each other in two or more nucleotides, to produce a library of recombinant genetic vaccine vectors;

(2) transfecting the library of recombinant vaccine vectors into a population of mammalian cells selected from the group consisting of peripheral blood T cells, T cell clones, freshly isolated monocytes/macrophages and dendritic cells;

(3) staining the cells for the presence of one or more cytokines and identifying cells which exhibit a cytokine staining pattern indicative of the desired immune response; and

(4) obtaining recombinant vaccine vector nucleic acid sequences from the cells which exhibit the desired cytokine staining pattern.

34. The method of claim 33, wherein the desired immune response is a T_H1 response and the cells exhibit high levels of either or both of IL-2 and IFN-γ but low levels of one or more of IL-4, IL-5 and IL-13.

35. The method of claim 33, wherein the cells are selected from the group consisting of monocytes, macrophages, and dendritic cells and the desired immune response is a high or low level of cytokine production by the cells.

36. The method of claim 35, wherein the cytokine expressed at a high level is one or more selected from the group consisting of IL-6, IL-10, IL-12 and TNF-α.

37. A method of improving the ability of a genetic vaccine vector to modulate an immune response, the method comprising:

(1) recombining at least first and second forms of a nucleic acid which comprise a genetic vaccine vector, wherein the first and second forms differ from each other in two or more nucleotides, to produce a library of recombinant genetic vaccine vectors;

(2) transfecting the library of recombinant genetic vaccine vectors into a population of antigen presenting cells; and

(3) isolating from the cells optimized recombinant genetic vaccine vectors which exhibit enhanced ability to modulate a desired immune response.

38. The method of claim 37, wherein the method further comprises:

(4) recombining at least one optimized recombinant vaccine vector with a further form of the genetic vaccine vector, which is the same or different from the first and second forms, to produce a further library of recombinant genetic vaccine vectors;

(5) transfecting the further library of recombinant genetic vaccine vectors into a population of antigen presenting cells;

(6) identifying optimized recombinant genetic vaccine vectors which exhibit enhanced ability to modulate a desired immune response; and

(7) repeating (4) through (6), as necessary, to obtain a further optimized recombinant genetic vaccine vector which has a further enhanced ability to modulate a desired immune response.

39. The method of claim 37, wherein the antigen presenting cell is selected from the group consisting of a dendritic cell, a B lymphocyte, a monocyte, a macrophage cell, and a Langerhans cell.

40. The method of claim 37, wherein the optimized recombinant genetic vaccine vectors exhibit improved ability to enter an antigen presenting cell and are obtained by:

after the transfection step, washing the cells to remove vectors which did not enter an antigen presenting cell;

culturing the cells for a predetermined time after transfection;

lysing the antigen presenting cells; and

isolating the optimized recombinant genetic vaccine vector from the cell lysate.

41. The method of claim 37, wherein APCs that contain an optimized recombinant genetic vaccine vectors are identified by detecting expression of a marker gene that is included in the vectors.

42. The method of claim 41, wherein the marker gene encodes a cell surface antigen.

43. The method of claim 42, wherein expression of the marker gene is detected by flow cytometric cell sorting.

44. The method of claim 37, wherein the genetic vaccine vector comprises a nucleotide sequence that encodes an immunogenic antigen and optimized recombinant genetic vaccine vectors are identified by:

transfecting individual library members into separate cultures of antigen presenting cells;

co-culturing transfected APCs with T lymphocytes obtained from the same individual as the APCs; and

identifying transfected APC cultures which are capable of inducing a T lymphocyte response.

45. The method of claim 44, wherein the T lymphocyte response is selected from the group consisting of increased T lymphocyte proliferation, increased T lymphocyte-mediated cytolytic activity against a target cell, and increased cytokine production.

46. The method of claim 45, wherein the genetic vaccine vector is capable of inducing a T_H1 response as evidenced by the transfected APCs inducing a T lymphocyte response that involves one or more of proliferation, IL-2 production, and interferon-γ production.

47. The method of claim 44, wherein the optimized recombinant genetic vaccine vectors are identified by its improved capacity to induce an immune response in a test animal, wherein the immune response is selected from the group consisting of:

improved protection of the test animal against challenge infection;

improved production of specific antibodies in the test animal; and

improved activation of T lymphocytes in the test animal.

48. The method of claim 47, wherein the test animal is a mouse or a monkey.

49. The method of claim 44, wherein T lymphocytes are selected from the group consisting of $CD4^+$ T lymphocytes, $CD8^+$ T lymphocytes, and a mixture thereof.

50. The method of claim 37, wherein the genetic vaccine vector comprises a nucleotide sequence that encodes an antigen and optimized recombinant vaccine vectors are identified by:

injecting the library of recombinant genetic vaccine vectors into a test animal;

obtaining lymphatic cells from the test animal; and

recovering recombinant genetic vaccine vectors from the lymphatic cells, wherein the recovered recombinant genetic vaccine vectors exhibit improved ability to enter lymphatic cells.

51. The method of claim 50, wherein the lymphatic cells are dendritic cells.

52. The method of claim 50, wherein the antigen is a cell surface antigen and prior to isolating the optimized recombinant genetic vaccine vectors cells that contain an optimized recombinant vector are purified by binding to an affinity reagent which selectively binds to the cell surface antigen.

53. A method of obtaining a recombinant genetic vaccine vector which has an enhanced ability to induce a desired immune response in a mammal upon administration to the skin of the mammal, the method comprising:

(1) recombining at least first and second forms of a nucleic acid which comprise a genetic vaccine vector, wherein the first and second forms differ from each other in two or more nucleotides, to produce a library of recombinant genetic vaccine vectors;

(2) topically applying the library of recombinant genetic vaccine vectors to skin of a mammal;

(3) identifying vectors that induce an immune response; and

(4) recovering genetic vaccine vectors from the skin cells which contain vectors that induce an immune response.

54. The method of claim 53, wherein the immune response is a protective immune response.

55. The method of claim 53, wherein the immune response is a CTL response, a T helper cell response, or an antibody response.

56. A method of inducing an immune response in a mammal, the method comprising topically applying to skin of the mammal a genetic vaccine vector, wherein the genetic vaccine vector is optimized for topical application through use of DNA shuffling.

57. The method of claim 56, wherein the genetic vaccine vector is administered as a formulation selected from the group consisting of a transdermal patch, a cream, naked DNA, a mixture of DNA and a transfection-enhancing agent.

58. The method of claim 57, wherein the transfection-enhancing agent is one or more agents selected from the group consisting of a lipid, a liposome, a protease, and a lipase.

59. The method of claim 56, wherein the genetic vaccine vector is administered after pretreatment of the skin by abrasion or hair removal.

60. A method of obtaining an optimized genetic vaccine component that confers upon a genetic vaccine containing the component an enhanced ability to induce or inhibit apoptosis of a cell into which the vaccine is introduced, the method comprising:

(1) recombining at least first and second forms of a nucleic acid which comprise a nucleic acid that encodes an apoptosis-modulating polypeptide, wherein the first and second forms differ from each other in two or more nucleotides, to produce a library of recombinant nucleic acids;

(2) transfecting the library of recombinant nucleic acids into a population of mammalian cells;

(3) staining the cells for the presence of a cell membrane change which is indicative of apoptosis initiation; and

(4) obtaining recombinant apoptosis-modulating genetic vaccine components from the cells which exhibit the desired apoptotic membrane changes.

61. The method of claim 60, wherein the genetic vaccine component has an enhanced ability to induce apoptosis and the nucleic acids encode an apoptosis-inducing polypeptide.

62. The method of claim 61, wherein the apoptosis-inducing polypeptide is a Caspases polypeptide or a Fas polypeptide.

63. The method of claim 60, wherein the genetic vaccine component has an enhanced ability to inhibit apoptosis and the nucleic acids encode an apoptosis-inhibiting polypeptide.

64. The method of claim 63, wherein the apoptosis-inhibiting polypeptide is Bcl-2 or another Bcl-2 family member.

65. The method of claim 60, wherein the cell membrane change which is indicative of apoptosis initiation is translocation of phospholipid phosphatidylserine (PS) from the inner to the outer leaflet of the plasma membrane.

66. The method of claim 65, wherein the PS translocation is detected by increased or decreased binding of Annexin V.

67. A method of obtaining a genetic vaccine component that confers upon a genetic vaccine reduced susceptibility to a CTL immune response in a host mammal, the method comprising:

(1) recombining at least first and second forms of a nucleic acid which comprises a gene that encodes an inhibitor of a CTL immune response, wherein the first and second forms differ from each other in two or more nucleotides, to produce a library of recombinant CTL inhibitor nucleic acids;

(2) introducing genetic vaccine vectors which comprise the library of recombinant CTL inhibitor nucleic acids into a plurality of human cells;

(3) selecting cells which exhibit reduced MHC class I molecule expression; and

(4) obtaining optimized recombinant CTL inhibitor nucleic acids from the selected cells.

68. The method of claim 67, wherein the method further comprises:

(5) recombining at least one recombinant CTL inhibitor nucleic acid with a further form of the gene that encodes an inhibitor of a CTL immune response, which

is the same or different from the first and second forms, to produce a further library of recombinant CTL inhibitor nucleic acids;

(6) introducing genetic vaccine vectors which comprise the library of recombinant CTL inhibitor nucleic acids into a plurality of human cells; and

(7) selecting cells which exhibit reduced MHC class I molecule expression, wherein the selected cells comprise recombinant genetic vaccine vectors which exhibit reduced susceptibility to a CTL immune response in a host mammal; and

(8) repeating (5) through (7), as necessary, to obtain a further optimized recombinant CTL inhibitor genetic vaccine component that confers upon a genetic vaccine a further reduced susceptibility to a CTL immune response in a host mammal.

69. The method of claim 67, wherein the nucleic acid comprises a gene that encodes an inhibitor of MHC class I-mediated antigen presentation.

70. The method of claim 69, wherein the gene is selected from the group consisting of US2, US3, US6 and US11 genes of cytomegalovirus, a gene encoding adenoviral E3 protein, a gene encoding herpes simplex ICP47 protein, and a gene encoding a tapasin antagonist.

71. The method of claim 67, wherein the genetic vaccine comprises a viral vector.

72. A method of obtaining a genetic vaccine component that confers upon a genetic vaccine reduced susceptibility to a CTL immune response in a host mammal, the method comprising:

(1) recombining at least first and second forms of a nucleic acid which comprises a gene that encodes an inhibitor of a CTL immune response, wherein the first and second forms differ from each other in two or more nucleotides, to produce a library of recombinant CTL inhibitor nucleic acids;

(2) introducing viral vectors which comprise the library of recombinant CTL inhibitor nucleic acids into mammalian cells;

(3) identifying mammalian cells which express a marker gene included in the viral vectors a predetermined time after introduction, wherein the identified cells are resistant to a CTL response; and

(4) recovering as the genetic vaccine component the recombinant CTL inhibitor nucleic acids from the identified cells.

73. The method of claim 72, wherein the genetic vaccine comprises a viral vector that is selected from the group consisting of papillomavirus, adenovirus, and retrovirus.